(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 574 769 B1

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
17.04.1996 Bulletin 1996/16

(51) Int Cl.[6]: **C07D 257/04**, G01N 33/52, C07D 417/04, C07F 9/6524

(21) Application number: **93108863.7**

(22) Date of filing: **02.06.1993**

(54) **Use of specific counteranions to modify the solubility of tetrazolium salts**

Verwendung spezifischer gegenanionen zum Verändern der Löslichkeit von Tetrazoliumsalzen

Utilisation de contre-ions spécifiques pour modifier la solubilité de sels de tetrazolium

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE

(30) Priority: **15.06.1992 US 898317**

(43) Date of publication of application:
22.12.1993 Bulletin 1993/51

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(56) References cited:
EP-A- 0 100 217
US-A- 3 655 382
US-A- 4 221 864
US-A- 4 334 071
US-A- 5 036 000

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

EP 0 574 769 B1

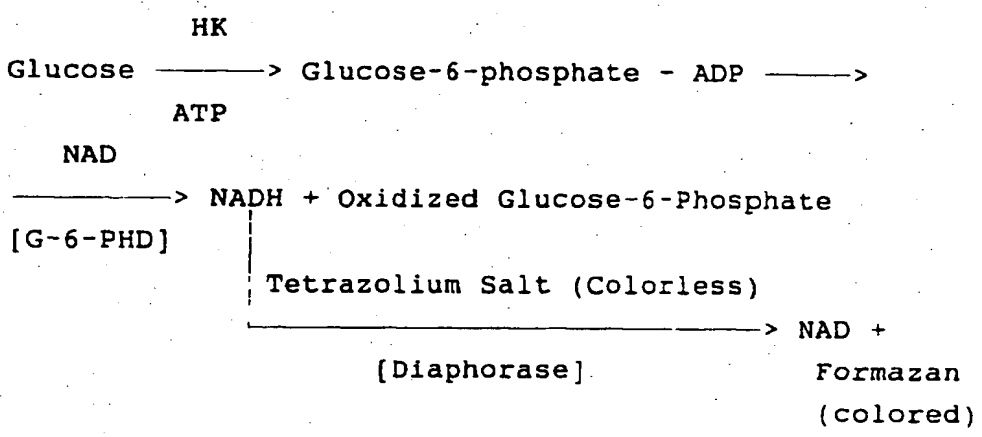
Printed by Jouve, 75001 PARIS (FR)

Description

BACKGROUND OF THE INVENTION

Tetrazolium salts, such as 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium (INT), are very useful in the measurement of analytes which can be converted to an equivalent concentration of NADH due to the reduction of the tetrazolium salt to its corresponding formazan which reduction can be accurately measured by colorimetric means.

A typical reagent system for determining glucose concentration in body fluids is based on reductive chemistry wherein the primary components are hexokinase (HK), adenosine triphosphate (ATP), glucose-6-phosphate dehydrogenase (G-6-PDH), diaphorase, nicotinamideadenine dinucleotide (NAD) and a tetrazolium salt as indicator. In operation, hexokinase catalyzes the reaction in which, in the presence of glucose, a phosphate radical is taken from ATP thereby converting it to adenosine diphosphate to form glucose-6-phosphate which is oxidized in the presence of NAD and G-6-PDH thereby reducing NAD to NADH. The NADH, in the presence of diaphorase as electron acceptor, reduces the colorless tetrazolium salt to its colored formazan counterpart thereby providing a detectable response. The reaction steps, as represented by the following scheme, represent the determination of NADH as an indirect means of determining the glucose concentration in the test sample:



The utility of tetrazolium salts in such systems for detecting such analytes is proportional to their solubility in water or suitable organic solvents. This is particularly true in the case of dry reagent diagnostic test devices, such as those in which a tetrazolium salt is dissolved in a polar organic solvent for impregnation into a carrier matrix such as paper or a polymer matrix or dissolved in an aqueous solution of a film forming polymer such as gelatin. Tetrazolium salt indicators are typically used with gelatin film and other dry reagent formulations which employ diaphorase or a chemical mediator in the color generating step. An adequate amount of indicator must be present to completely consume the reducing equivalents that originate from the influx of an analyte such as glucose. In most cases, in order to obtain a reasonably thin coating of the film forming polymer and to provide a sufficient supply of the indicator within the porous matrix, the concentration of indicator must be in the range of 0.05M to 0.15M or more.

United States patent 1,892,019 discloses the increased water solubility of benzylmorphine after it is reacted with alkyl sulphonic acid, e.g. methane or ethanesulfonic acid, by formation of the corresponding salt. U.S. patent 4,334,071 discloses the enhancement of the solubility of 17-cyclobutylmethyl-3-hydroxy-8β-methyl-6-methylene morphinane by converting its chloride salt to the corresponding methanesulfonate.

United States patent 3,655,382 discloses tetrazolium thiazolium salts in which the counteranion can be chloride, iodide, bromide, thiocyanate, thiosulfate, sulfate, paratoluenesulfonate, methylsulfate, ethyl sulfate, nitrate, acetate, perchlorate, perborate, sulfite, hydroxide or carbonate.

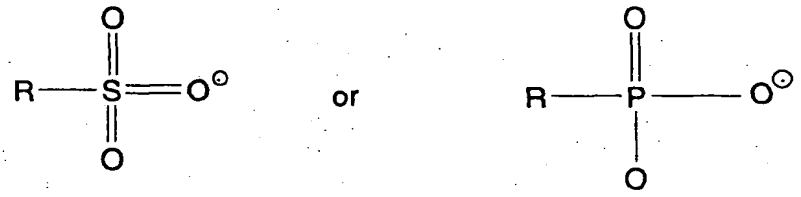
In United States patent 4,221,864 the patentees state that one of the objects of their invention is to provide a novel light sensitive photographic material containing a tetrazolium compound. They point out that this and other objects can be attained by preparing a photographic material which comprises a support and at least one light sensitive silver halide layer and another hydrophylic colloidal layer coated on the support, one of which layer contains a tetrazolium salt. They point out that where the salt of a tetrazolium compound is used as a non-diffusible ingredient, such a salt can be synthesized by reacting a tetrazolium cation with an anion capable of making the selected compound non-diffusible. Counteranions such as those derived from higher alkylbenzenesulfonic acids, e. g. dodecylbenzenesulfonic acid or a higher alkyl sulfuric acid ester such as lauryl sulfate are disclosed.

United States Patent 5,036,000 discloses that, in a system for quantitative colorimetric analysis of biological fluids involving NAD(P)H and a chromogen, tetrazolium salts are conveniently used. This reference goes on to note that the

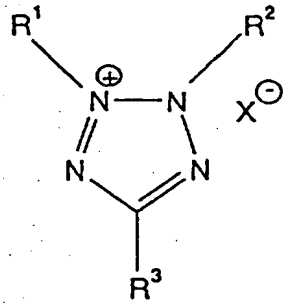
concentration of the tetrazolium salt is rather limited by its solubility.

SUMMARY OF THE INVENTION

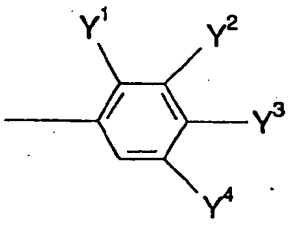
The present invention involves certains salts of tetrazolium compounds which exhibit unexpectedly high solubility in polar solvents. These salts include a counteranion of the formula:



Also inclused within the scope of this invention is a diagnostic test device comprising a reagent system incorporated into a carrier matrix containing one or more of the sulfonate and/or phosphate tetrazolium salts,
wherein R is a straight or branched alkyl group of form 1 to 7 carbon atoms or phenyl and wherein the tetrazolium cation is represented by the formula:



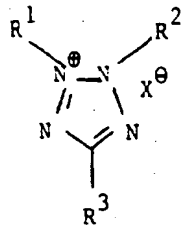
wherein $X^{\ominus}$ is the counteranion, R^1 and R^3 are phenyl groups and R^2 is phenyl or 2-thiazol wherein the phenyl groups are unsubstituted or substituted as represented by the formula



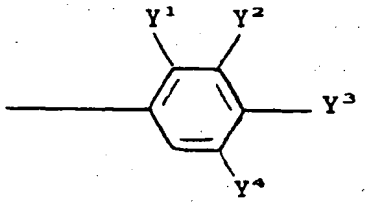
wherein the Y group, which can be the same of different are alkoxy, aryloxy, alkyl, amido, alkylamido, alkylthio, arylthio, halo, hydrogen, hydroxy, carbamoyl, carbalkoxy, carboxyl, cyano, nitro, sulfo, sulfoamido, sulfanoyl, trialkylamino or ureido.

DESCRIPTION OF THE INVENTION

The tetrazolium salts of the present invention can be represented by the formula:

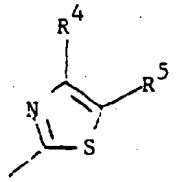


wherein $X^{\ominus}$ is the counteranion as defined above, R^1 and R^3 are phenyl groups and R^2 is phenyl or 2-thiazolyl. The phenyl and optional thiazol groups can be substituted or unsubstituted. More specifically, R^1, R^3 and optionally R^2 can be represented by, but are not limited to, the formula:



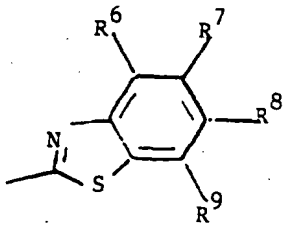
wherein the Y groups (Y^1, Y^2, Y^3 or Y^4) which are the same or different can be, for example, alkoxy, aryloxy, alkyl, amido, alkylamido, arylamido, alkylthio, arylthio, halo, hydrogen, hydroxy, carbamoyl, carbalkoxy, carboxyl, cyano, nitro, sulfo, sulfonamido, sulfamoyl, trialkylamino or ureido groups.

When R_2 is a thiazole group, it can be unsubstituted or substituted. For example, where the thiazole group is represented by the formula:



where R^4 and R^5 are hydrogen or some other substituent.

In a preferred embodiment of the present invention, the R^1 and R^3 moieties of the tetrazolium salt are as described above and R^2 is a thiazole group in which R^4 and R^5 together form a benzo ring which is substituted or unsubstituted; R^4 is carboxyl, carbalkoxy, carbamoyl, or cyano and R^5 is alkyl or chloro; R^4 is alkyl or aryl and R^5 is carboxyl, carbalkoxy, carbaryloxy, carbamoyl or cyano; R^4 is di- or trifluoroalkyl wherein the fluoro substituents are on the carbon adjacent to the thiazolyl residue; or one or both of R^4 and R^5 are substituted or unsubstituted phenyl, and if only one is substituted phenyl, the other is hydrogen or alkyl. Among those tetrazolium cations which are particularly useful in the context of the present invention are those in which R^4 and R^5 together form a benzo ring to give a benzothiazole residue having the formula:



wherein

(i) R^6 and R^7 or R^7 and R^8 or R^8 and R^9 together form a benzo or cyclohexyl ring that is unsubstituted or substituted with alkoxy, aryloxy, alkyl, amido, alkylamido, arylamido, alkylthio, arylthio, amino, carbamoyl, carbalkoxy, cyano, halo, hydroxyl, sulfo, sulfonamido, sulfamoyl, trialkylammonio, or ureido, and wherein the others, same or different,

are hydrogen, alkoxy, aryloxy, alkyl, amido, alkylamido, arylamido, alkylthio, arylthio, amino, carbamoyl, carbalkoxy, cyano, halo, hydroxyl, sulfo, sulfonamido, sulfamoyl, trialkylammonio, or ureido, provided that where R^7 and R^8 together form a benzo or cyclohexyl ring, R^6 is not hydrogen, or

(ii) one or more of R^6, R^7, R^8, and R^9 is alkoxy, aryloxy, alkyl, amido, alkylamido, arylamido, alkylthio, arylthio, amino, carbamoyl, carbalkoxy, cyano, halo, hydroxyl, sulfo, sulfonamido, sulfamoyl, trialkylammonio, or ureido, and the others, if any, are hydrogen.

The salts of the present invention are most conveniently prepared by interaction of less soluble salts of the tetrazolium compound with an anion exchange resin which is converted to its alkyl or benzene sulfonate or phosphonate form. This procedure is preferably carried out in the presence of an ion exchange resin due to the ease of purification which is rendered by this technique. Thus, by using the ion exchange resin one can simply stir the tetrazolium salt in a slurry of the resin followed by filtration, concentration and crystallization to obtain the pure salt. Alternative procedures which involve stirring the less soluble tetrazolium salt with the alkyl or benzene sulfonic or phosphoric acid or their salts can also be employed. However, when an ion exchange resin is used the excess sulfonate or phosphate tetrazolium salt is attached to the resin and can be separated from the reaction mass by filtration.

The following examples illustrate the general procedure for preparation of the tetrazolium salts of the present invention and their inclusion in analytical test devices.

EXAMPLE I

A. Preparation of ion exchange resin (RSO_3^- form).

Sulfonic acid is added to 20 g of Amberlite IRA-400 (-OH) ion exchange resin in 60 mL of water until a pH of 1.5 is achieved. The mixture is filtered and washed with 100 mL of water followed by a second washing with 100 mL of methanol.

B. Preparation of tetrazolium sulfonates.

A slurry of 5.5 g of tetrazolium salt, e.g. the tetrafluoroborate, and 50 g of moist resin is stirred in 300 mL of methanol for 2-4 hours. In cases where the tetrazolium salt's poor solubility inhibits the exchange, the mixture is warmed to 40°C. The mixture is filtered and then concentrated to a gum like residue whereupon the product precipitates after stirring with ethyl acetate.

C. Preparation of tetrazolium bromides.

A slurry of 2 g of the tetrazolium tetrafluoroborate is stirred overnight with 50 mL of 48% hydrobromic acid. The mixture is then filtered and washed with 200 mL of water to yield the tetrazolium bromide.

D. Preparation of tetrazolium tetrafluoroborates.

These salts are prepared by stirring the appropriate formazan with isoamylnitrate in the presence of 48% fluoroboric acid in acetic acid and filtering the product. Optionally, if the product does not precipitate, ether is added to force precipitation. Nitrate salts are prepared in a similar manner in the absence of fluoroboric acid.

E. Solubility testing.

Approximately 10 mg of the tetrazolium salt is measured out and the solvent added in increments of 25 µL until the salt dissolves. When it becomes apparent that a particular compound is only marginally soluble, the volume of solvent increments is increased to 50 and then to 100 µL.

The results of this solubility testing are set out in Table I in which the following abbreviations are used:

INT	2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium
MTT	[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium
DCT	2-(4-difluoromethyl-5-chlorothiazol-2-yl)-3-(3, 4,5-trimethoxyphenyl)-5-(3,4-methylenedioxyphenyl) tetrazolium
DCMT	2-(4-difluoromethyl-5-chlorothiazol-2-yl)-3-(2 methoxyphenyl) -5-(3,4-methylenedioxyphenyl) tetrazolium
MTM	2-(5-methoxynaphtho[1,2-d]thiazol-2-yl)-3-(3,4, 5-trimethoxyphenyl)-5-(4-methoxyphenyl) tetrazolium
Me	methyl

Et ethyl
Pr propyl
Bu butyl
Pe pentyl
Bz benzene

TABLE I

Compound	mp(°C)	Solubility (m mol mL)	
		Methanol	Water
INT Cl^-			3
INT $MeSO_3^-$	132-135		35
MTT Br^-		360	18
MTT $MeSO_3^-$	189-193	1150	>1,000
DCT Br^-	189-191	10	<1
DCT BF_4^-	239-241	<16	<0.5
DCT NO_3^-	184-185	22	2
DCT $MeSO_3^-$	129-131	>500	73
DCT $EtSO_3^-$	157-159	>600	26
DCT $PrSO_3^-$	174-176	>650	16
DCT $BzSO_3^-$	175-177	>550	
DCMT Br^-	167-169	24	<1
DCMT BF_4^-	231-233	12.9	2.7
DCMT $MeSO_3^-$	170-179	>760	248
DCMT $PrSO_3^-$	176-168	>760	23
DCMT $BuSO_3^-$	183-185	>700	15
DCMT $PeSO_3^-$	198-200	>700	2.6
MTM NO_3^-	251-252	7.6	
MTM $MeSO_3^-$	257-258	48	
MTM $PrSO_3^-$	241-141	100	
MTM $BzSO_3$	251-251	19	

From the data tabulated in Table I it can be determined that the conversion of the chloride salt of INT to its methanesulfonate increased its water solubility by a factor of greater than 10. The conversion of MTT bromide to the methane sulfonate provides an even greater increase in water solubility as well as increasing the salt's solubility in methanol. In the case of DCT, the bromide, tetrafluoroborate and nitrate salts are only marginally soluble in water and methanol whereas the methanesulfonate is highly soluble in both. As the size of the alkyl group increases, the water solubility decreases whereas solubility in methanol increases. The enhanced water solubility of these salts is significant since it facilitates the inclusion of adequate quantities of the tetrazolium salt into thin films of water soluble polymers such as gelatin. The high methanol solubility of the benzenesulfonate is also significant. The use of non-aqueous solvents in preparing the previously mentioned carrier matrix films is important because it enables one to deposit the indicator into the matrix from non-aqueous solutions wherein the liquid phase is a non-solvent for the reagent system used to create the detectable response. Typical carrier matrixes include bibulous materials such as filter paper or a nonbibulous material such as a membrane of a polymerized substance or a combination thereof. Accordingly, it is significant that methanol solubility of MTM is substantially increased by converting it to the methane- or propanesulfonate. The solubility of this tetrazolium compound decreases when it is converted to the benzenesulfonate but is still substantially greater than the nitrate. Conversely, no improvement in methanol solubility was observed for the paratoluene and naphthalene salts of these tetrazolium compounds.

Further reference to Table I reveals that the water and methanol solubility of DCMT is greatly enhanced by conversion of the bromide or tetrafluoroborate salts to the corresponding methanesulfonate. As the length of the alkyl chain increas-

es, water solubility declines while methanol solubility remains substantially unchanged.

The tetrazolium salts of the present invention are particularly suitable for use in analytical test devices of the type previously mentioned since the solubility of the salt can be tailored to the particular device being fabricated. For example if it is desired to impregnate a carrier matrix or a gelatin film with the tetrazolium salt from an aqueous solution, the organic moiety, R in the foregoing general formula, is lower alkyl, preferably methyl, in order to provide a tetrazolium salt with the requisite hydrophilic properties. In the manufacture of analytical devices where it is desirable to apply the various reagents from a solution other than that from which the tetrazolium salt is applied, the R group is selected to render the salt soluble in polar organic solvents, which are not good solvents for the other reagents, to facilitate application of the tetrazolium indicator from its solution in the polar organic solvent either before or after the other reagents have been applied to the substrate from their aqueous solution. In this manner premixing of the reagent system and the tetrazolium salt in a single solvent system can be avoided by tailoring the counteranion to the solvent system of choice. Methanol is a particularly good solvent for certain tetrazolium indicators wherein the R group in the counteranion is phenyl.

The preparation of a polymer matrix, analytical device using a tetrazolium salt of the present invention is illustrated by the following example.

EXAMPLE II

An 80 millimole/liter solution of 2-(4-difluoromethyl-5-chlorothiazol-2-yl)-3-(3,4,5 trimethoxyphenyl)5-(3,4-methylenedioxyphenyl) tetrazolium benzenesulfonate in methanol containing 0.75% Cremophor surfactant was prepared. A 500 foot strip of a 6 mil thick, 8.625 inch wide, zwitterionic charged nylon fabric was impregnated with 4 liters of the tetrazolium salt solution to cause saturation. Extraction of the fabric with methanol and determination of the indicator's concentration by HPLC spectroscopy indicated that it was present in the fabric at a concentration of 4-5 μ mole/in^2. After drying, the strip was treated with 5 1/2 liters of an aqueous solution containing 100 mM/L adenosine triphosphate. After the aqueous impregnation, 1 liter of the aqueous solution remained which was found to contain 3 mM of the tetrazolium salt which was extracted from the treated membrane. The solution also contained some formazan which was not quantified. Assuming that the formazan was also 3 mM, there was recovered an equivalent of 6 millimoles of the tetrazolium salt which had been extracted from the membrane during the aqueous impregnation, representing a loss of 1.875 percent.

4 L x 0.08 moles/L = 0.32 moles of tetrazolium salt impregnated into the membrane during first treatment.

1 L x 0.006 moles/L = 0.006 moles extracted during aqueous impregnation.

$$\frac{0.006}{0.320} = 1.875\%$$

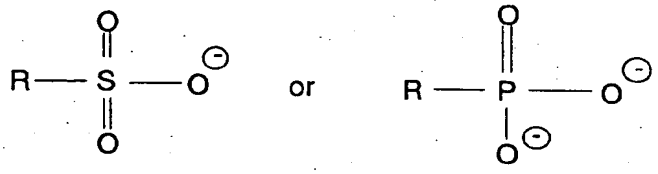
The amount of ATP in the membrane was determined to be between 82 and 92% of the theoretical level.

Impregnation of the fabric with methanolic, ethanolic or other alcoholic solutions of the indicator in which the counteranion was nitrate or tetrafluoroborate was unsuccessful due to the low solubility of these salts in alcohol. Concentrations of these salts comparable to that achieved with methanol was achieved using a 1:1 mixture of dimethylformamide and methanol. However, the use of dimethylformamide is undesirable on an industrial manufacturing scale since it is an established liver and kidney toxin. Furthermore, application of the tetrazolium salt from its methanol solution facilitates the use of a two dip procedure for applying the indicator in a first dip with the enzymes and other water soluble constituents of the reagent system in a second dip from their aqueous solutions without rehydrating the already deposited tetrazolium salt. By selecting a more hydrophilic organic radical, such as that of methanesulfonate, the tetrazolium salt is rendered water soluble so that the entire reagent system including the indicator can be applied in a single dip. Application from separate dips as in this example is preferred in order to minimize interaction between the reagents during the application of the reagent system to the substrate.

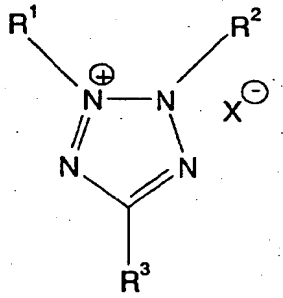
While the foregoing data illustrate the enhanced solubility of alkyl and benzenesulfonates, similar results can be achieved with the corresponding phosphonate salts. This is the case because the oxygen atoms on the phosphorous group improve water solubility through hydrogen bonding in a manner similar to those on the sulfonate. The organic group on the phosphonates is analogous to the alkyl or phenyl group of the sulfonates and can be manipulated in a similar manner to aid in the salt's solubility in various solvents.

Claims

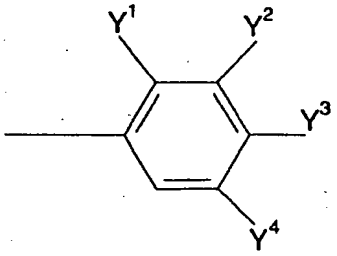
1. Sulfonate and phosphonate salts of tetrazolium compounds wherein the anion is represented by the formula:



wherein R is a straight or branched alkyl group of form 1 to 7 carbon atoms or phenyl and wherein the tetrazolium cation is represented by the formula:

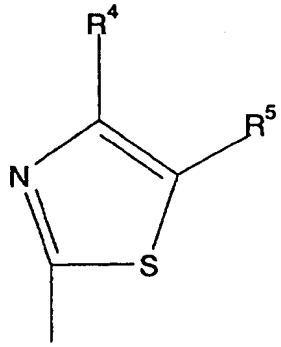


wherein $X^{\ominus}$ is the counter anion, R^1 and R^3 are phenyl groups and R^2 is phenyl or 2-thiazolyl wherein the phenyl groups are unsubstituted or substituted as represented by the formula



wherein the Y groups, which can be the same or different are alkoxy, aryloxy, alkyl, amido, alkylamido, arylamido, alkylthio, arylthio, halo, hydrogen, hydroxy, carbamoyl, carbalkoxy, carboxyl, cyano, nitro, sulfo, sulfoamido, sulfanoyl, trialkylamino or ureido.

2. The tetrazolium salts of Claim 1 wherein R^2 is substituted or unsubstituted thiazol characterized by the formula:

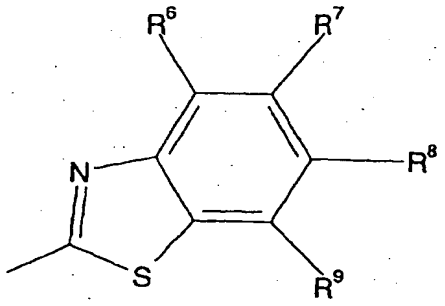


wherein R^4 and R^5 together form a benzo ring which is substituted or unsubstituted or R^4 is hydrogen, carboxyl, carbalkoxy, carbamoyl, or cyano and R^5 is hydrogen, alkyl or chloro or R^4 is alkyl or aryl and R^5 is carboxyl, carbalkoxy, carbaryloxy, carbamoyl or cyano or R^4 is di- or trifluoroalkyl wherein the fluoro substituents are on the

carbon adjacent to the thiazole residue; or one or both of R^4 and R^5 are substituted phenyl and if only one is substituted phenyl, the other is hydrogen or alkyl.

3. The tetrazolium salts of any of Claims 1 and 2 wherein R^4 is difluoromethyl and R^5 is chloro.

4. The tetrazolium salts of any of Claims 1 and 2 wherein R^4 and R^5 together form a benzo ring to provide a benzthiazole residue having the formula:



wherein

(i) R^6 and R^7 or R^7 and R^8 and R^9 together form a benzo or cyclohexyl ring that is unsubstituted or substituted with alkoxy, aryloxy, alkyl, carbamoyl, carbalkoxy, cyano, halo, hydroxyl, sulfo, sulfonamido, sulfamoyl, trialkylamino or ureido and where the others, which can be the same or different, are hydrogen, alkoxy, aryloxy, alkyl, amido, alkylamido, arylamido, alkylthio, arylthio, amino, carbamoyl, carbalkoxy, cyano, halo, hydroxyl, sulfo, sulfonamido, sulfamoyl, trialkylamino or ureido provided that when R^7 and R^8 together form a benzo or cyclohexyl ring, R^6 is not hydrogen, or

(ii) one or more of R^6, R^7, R^8 and R^9 is alkoxy, aryloxy, alkyl, amido, alkylamido, arylamido, alkylthio, arylthio, amino, carbamoyl, carbalkoxy, cyano, halo, hydroxyl, sulfo, sulfonamido, sulfamoyl, trialkylamino or ureido.

5. The tetrazolium salts of Claim 4 wherein the cation is 2-(4-difluoromethyl-5-chlorothiazol-2-yl)-3-(3,4,5-trimethoxyphenyl)-5-(3,4-methylenedioxyphenyl) tetrazolium or 2-(4-difluoromethyl-5-ch lorothiazol-2-yl)-3-(2-methoxyphenyl)-5-(3,4-methylenedioxyphenyl) tetrazolium.

6. A method of applying a tetrazolium salt to a matrix carrier material which comprises contacting said matrix earrier material with an aqueous or alcoholic solution of the tetrazolium salt according to any of Claims 1 - 5.

7. A method of preparing a dry reagent analytical device which involves:

a) contacting the matrix carrier material with a alcohol solution of a tetrazolium salt according to any one of Claim 1 - 5;

b) allowing the alcohol to evaporate;

c) contacting the resulting tetrazolium salt bearing matrix with an aqueous solution of a reagent system which, when contacted with an analyte, will undergo a series of reactions which result in the reduction of the tetrazolium salt to its corresponding formazan state, to deposit the individual components of the reagent solution into to matrix material without eluting a significant amount of the tetrazolium salt therefrom to provide, upon drying, an indicator/reagent system which upon contact with a solution of the analyte will provide a detectable response as the colorless tetrazolium salt is reduced to its corresponding colored formazan.

8. Use of the tetrazolium salt as defined in claims 1 - 5 in the determination of an analyte.

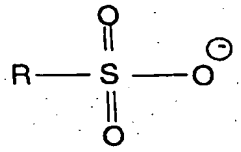
9. A diagnostic reagent containing a tetrazolium salt according to any one of claims 1 - 5.

10. A diagnostic test device comprising a reagent system incorporated into a carrier matrix containing one or more of

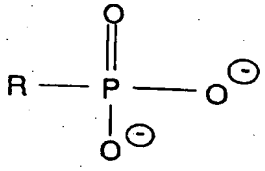
the tetrazolium salts according to any one of claim 1 - 5.

Patentansprüche

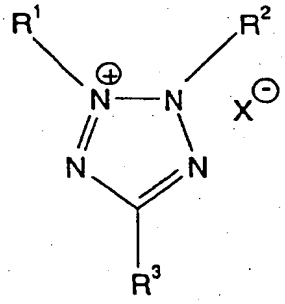
1. Sulfonat- und Phosphonatsalze von Tetrazoliumverbindungen, worin das Anion durch die Formel dargestellt ist:



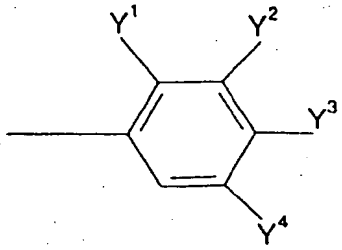
oder



worin R eine gerade oder verzweigte Alkylgruppe mit 1 bis 7 Kohlenstoffatomen oder eine Phenylgruppe ist, und worin das Tetrazolium-Kation durch die Formel dargestellt ist:

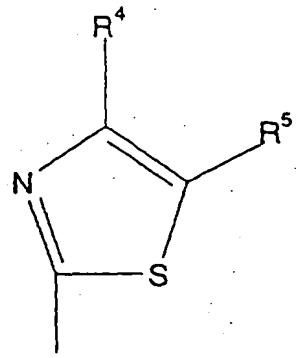


worin X^- das Gegenanion, R^1 und R^3 Phenylgruppen und R^2 eine Phenyl- oder 2-Thiazolylgruppe sind, worin die Phenylgruppen unsubstituiert oder substituiert und durch die Formel dargestellt sind:



worin die Y-Gruppen, die gleich oder verschieden sein können, Alkoxy-, Aryloxy-, Alkyl-, Amido-, Alkylamido-, Arylamido-, Arylthio-Gruppen, Halogen, Wasserstoff, Hydroxy-, Carbamoyl-, Carbalkoxy-, Carboxyl-, Cyano-, Nitro-, Sulfo-, Sulfoamido-, Sulfamoyl-, Trialkylamino- oder Ureido-Reste sind.

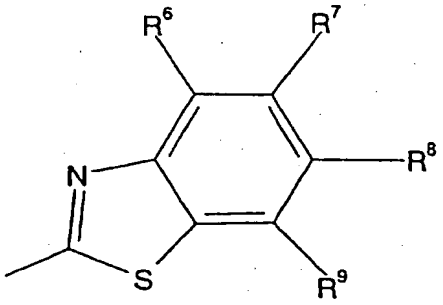
2. Tetrazoliumsalze gemäß Anspruch 1, worin R^2 eine substituierte oder unsubstituierte Thiazolgruppe ist, die durch die Formel gekennzeichnet ist:



worin R^4 und R^5 zusammen einen Benzolring bilden, der substituiert oder unsubstituiert ist, oder worin R^4 Wasserstoff, eine Carboxyl-, Carbalkoxy-, Carbamoyl- oder Cyano-Gruppe und R^5 Wasserstoff, eine Alkylgruppe oder Chlor oder R^4 eine Alkyl- oder Arylgruppe und R^5 eine Carboxyl-, Carbalkoxy-, Carbaryloxy-, Carbamoyl- oder Cyanogruppe oder R^4 eine Di- oder Trifluoralkylgruppe sind, worin sich die Fluor-Substituenten am zum Thiazolrest benachbarten Kohlenstoffatom befinden, oder worin einer oder beide der Reste R^4 und R^5 substituierte Phenylgruppen sind, und wenn nur einer eine substituierte Phenylgruppe ist, der andere Rest Wasserstoff oder eine Alkylgruppe ist.

3. Tetrazoliumsalze gemäß Anspruch 1 oder 2, worin R^4 der Difluormethylrest und R^5 Chlor sind.

4. Tetrazoliumsalze gemäß Anspurch 1 oder 2, worin R^4 und R^5 zusammen einen Benzolring bilden, um einen Benzthiazolrest zu ergeben, der die Formel aufweist:



worin

(i) R^6 und R^7 oder R^7 und R^8 oder R^8 und R^9 zusammen einen Benzolring oder Cyclohexylring bilden, die unsubstituiert oder mit einem Alkoxy-, Aryloxy-, Alkyl-, Carbamoyl-, Carbalkoxy-, Cyano-, Halo-, Hydroxyl-, Sulfo-, Sulfonamido-, Sulfamoyl-, Trialkylammonio- oder Ureidorest substituiert sind und worin die weiteren Reste, gleich oder verschieden, Wasserstoff, Alkoxy-, Aryloxy-, Alkyl-, Amido-, Alkylamido-, Arylamido-, Alkylthio-, Arylthio-, Amino-, Carbamoyl-, Carbalkoxy-, Cyano-, Halo-, Hydroxyl-, Sulfo-, Sulfonamido-, Sulfamoyl-, Trialkylammonio- oder Ureidoreste sind, mit der Maßgabe, daß, wenn R^7 und R^8 zusammen einen Benzol- oder Cyclohexylring bilden, R^6 nicht Wasserstoff ist, oder worin

(ii) einer oder mehrere der Reste R^6, R^7, R^8 und R^9 Alkoxy-, Aryloxy-, Alkyl-, Amido-, Alkylamido-, Arylamido-, Alkylthio- Arylthio-, Amino-, Carbamoyl-, Carbalkoxy-, Cyano-, Halo-, Hydroxyl-, Sulfo-, Sulfonamido-, Sulfamoyl-, Trialkylammonio- oder Ureidoreste sind.

5. Tetrazoliumsalze gemäß Anspruch 4, worin das Kation das 2-(4-Difluormethyl-5-chlorthiazol-2-yl)-3-(3,4,5-trimethoxyphenyl)-5-(3,4-methylendioxyphenyltetrazolium)- oder das 2-(4-Difluormethyl-5-chlorthiazol-2-yl)-3-(2-methoxyphenyl)-5-(3,4-methylendioxyphenyl)tetrazolium-Kation ist.

6. Verfahren zur Aufbringung eines Tetrazoliumsalzes auf ein Matrix-Trägermaterial, wobei man das genannte Matrix-Trägermaterial mit einer wässrigen oder alkoholischen Lösung des Tetrazoliumsalzes gemäß einem der Ansprüche 1 bis 5 in Kontakt bringt.

7. Verfahren zur Herstellung einer analytischen Vorrichtung mit trockenem Reagens, wobei man:

a) das Matrix-Trägermaterial mit einer Alkohol-Lösung eines Tetrazoliumsalzes gemäß einem der Ansprüche 1 bis 5 in Kontakt bringt,

b) den Alkohol verdampfen läßt und

c) die das sich ergebende Tetrazoliumsalz aufweisende Matrix mit einer wässrigen Lösung eines Reagens-Systems, das, bei Kontakt mit einem Analyt, eine Reihe von Reaktionen mit dem Ergebnis der Reduktion des Tetrazoliumsalzes zu seinem entsprechenden Formazan-Zustand einzugehen befähigt ist, in Kontakt bringt, um die jeweiligen Komponenten der Reagens-Lösung im Matrixmaterial abzuscheiden, ohne daraus eine signifikante Menge des Tetrazoliumsalzes wieder zu eluieren, um, nach Trocknung, ein Indikator/Reagens-System breitzustellen, das bei Kontakt mit einer Lösung des Analyt eine nachweisbare Reaktion ergibt, und zwar dadurch, daß das farblose Tetrazoliumsalz zu seinem entsprechenden gefärbten Formazan reduziert wird.

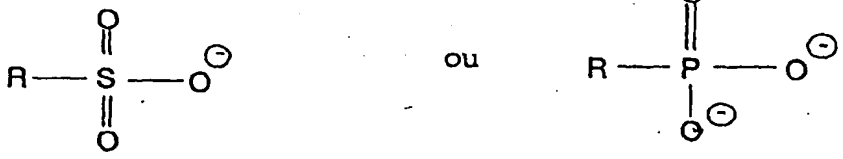
8. Verwendung des Tetrazoliumsalzes gemäß einem der Ansprüche 1 bis 5 zur Bestimmung eines Analyt.

9. Diagnostisches Reagens, enthaltend ein Tetrazoliumsalz gemäß einem der Ansprüche 1 bis 5.

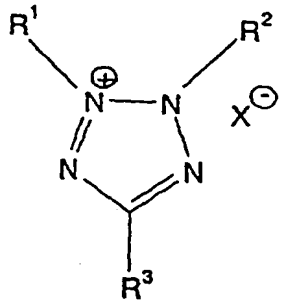
10. Diagnostische Testvorrichtung, umfassend ein Reagens-System, das in eine Trägermatrix eingebracht ist, die eines oder mehrere der Tetrazoliumsalze gemäß einem der Ansprüche 1 bis 5 enthält.

Revendications

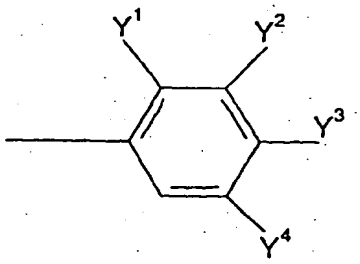
1. Sels sulfoniques et phosphoniques de composés de tétrazolium, dans lesquels l'anion est représenté par la formule :



où R est un groupe alkyle linéaire ou ramifié ayant 1 à 7 atomes de carbone ou un groupe phényle et le cation tétrazolium est représenté par la formule :

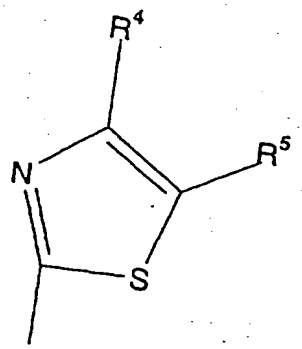


dans laquelle $X^{\ominus}$ est l'anion complémentaire, R^1 et R^3 sont des groupes phényle et R^2 est un groupe phényle ou 2-thiazolyle, les groupes phényle étant non substitués ou substitués comme représenté par la formule



dans laquelle les groupes Y, qui peuvent être identiques ou différents, sont des radicaux alkoxy, aryloxy, alkyle, amido, alkylamido, arylamido, alkylthio, arylthio, halogéno, hydrogène, hydroxy, carbamoyle, carbalkoxy, carboxyle, cyano, nitro, sulfo, sulfamido, sulfanoyle, trialkylamino ou uréido.

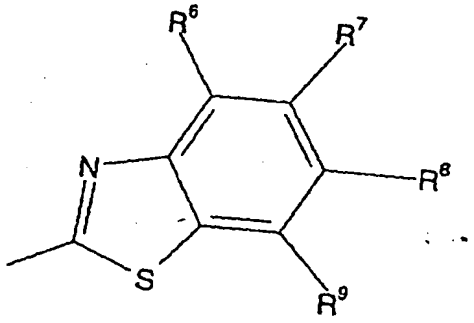
2. Sels de tétrazolium suivant la revendication 1, dans lesquels R^2 est un thiazole substitué ou non substitué, caractérisé par la formule :



dans laquelle R^4 et R^5 forment ensemble un noyau benzénique qui est substitué ou non substitué ou bien R^4 est de l'hydrogène, un radical carboxyle, carbalkoxy, carbamoyle ou cyano et R^5 est de l'hydrogène, un radical alkyle ou chloro, ou bien R^4 est un radical alkyle ou aryle et R^5 est un radical carboxyle, carbalkoxy, carbaryloxy, carbamoyle ou cyano, ou bien R^4 est un radical di- ou trifluoralkyle dont les substituants fluoro sont portés par l'atome de carbone adjacent au résidu thiazole ; ou bien l'un de R^4 et R^5 ou les deux sont des groupes phényle substitués et, si un seul est un groupe phényle substitué, l'autre est de l'hydrogène ou un groupe alkyle.

3. Sels de tétrazolium suivant l'une quelconque des revendications 1 et 2, dans lesquels R^4 est un groupe difluorométhyle et R^5 est un radical chloro.

4. Sels de tétrazolium suivant l'une quelconque des revendications 1 et 2, dans lesquelles R^4 et R^5 forment ensemble un noyau benzénique pour réaliser un résidu benzothiazole répondant à la formule :



dans laquelle (i) R^6 et R^7, ou bien R^7 et R^8 et R^9 forment ensemble un noyau benzénique ou cyclohexyle qui est

non substitué ou substitué avec un radical alkoxy, aryloxy, alkyle, carbamoyle, carbalkoxy, cyano, halogéno, hydroxyle, sulfo, sulfonamido, sulfamoyle, trialkylamino ou uréido et les autres, qui peuvent être identiques ou différents, représentent de l'hydrogène, un radical alkoxy, aryloxy, alkyle, amide, alkylamido, arylamido, alkylthio, arylthio, amino, carbamoyle, carbalkoxy, cyano, halogéno, hydroxyle, sulfo, sulfonamido, sulfamoyle, trialkylamino ou uréido, sous réserve que, lorsque R^7 et R^8 forment ensemble un noyau benzénique ou cyclohexyle, R^6 ne soit pas de l'hydrogène, ou bien (ii) l'un au moins de R^6, R^7, R^8 et R^9 est un radical alkoxy, aryloxy, alkyle, amido, alkylamido, arylamido, alkylthio, arylthio, amino, carbamoyle, carbalkoxy, cyano, halogéno, hydroxyle, sulfo, sulfonamido, sulfamoyle, trialkylamino ou uréido.

5. Sels de tétrazolium suivant la revendication 4, dans lesquels le cation est le 2-(4-difluorométhyl-5-chlorothiazole-2-yl)-3-(3,4,5-triméthoxyphényl)-5-(3,4-méthylènedioxyphényl)tétrazolium ou le 2-(4-difluorométhyl-5-chlorothiazole-2-yl)-3-(2-méthoxyphényl)-5-(3,4-méthylènedioxyphényl)tétrazolium.

6. Procédé d'application d'un sel de tétrazolium à une matrice de support, qui comprend la mise en contact de cette matrice de support avec une solution aqueuse ou alcoolique du sel de tétrazolium suivant l'une quelconque des revendications 1 à 5.

7. Procédé de préparation d'un disposition analytique à réactif à l'état sec, qui implique :

a) la mise en contact de la matrice de support avec une solution alcoolique d'un sel de tétrazolium suivant l'une quelconque des revendications 1 à 5
b) l'évaporation de l'alcool ;
c) la mise en contact de la matrice résultante portant le sel de tétrazolium avec une solution aqueuse d'un système réactif qui, au contact d'un analyte, subit une série de réactions qui aboutissent à la réduction du sel de tétrazolium en son état de formazane correspondant, pour déposer les composants individuels de la solution de réactif dans la matrice sans en éluer une quantité importante du sel de tétrazolium pour réaliser, par séchage, un système indicateur/réactif qui, au contact d'une solution de l'analyte, donne une réponse détectable à mesure que le sel de tétrazolium incolore est réduit en son formazane coloré correspondant.

8. Utilisation du sel de tétrazolium tel que défini dans les revendications 1 à 5 dans le dosage d'un analyte.

9. Réactif diagnostique contenant un sel de tétrazolium suivant l'une quelconque des revendications 1 à 5.

10. Dispositif d'essai diagnostique comprenant un système réactif incorporé à une matrice contenant un ou plusieurs des sels de tétrazolium suivant l'une quelconque des revendications 1 à 5.